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DOI: <https://doi.org/10.17236/sat00012>

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ZORA URL: <https://doi.org/10.5167/uzh-122090>

Journal Article

Published Version

Originally published at:

Ruf, J; Johler, Sophia; Merz, Axel; Stalder, Ueli; Hässig, M (2015). Success of interventions in mastitis problems with *Staphylococcus aureus* after the introduction of an automatic milking system. *Schweizer Archiv für Tierheilkunde*, 157(3):153-156.

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Success of interventions in mastitis problems with *Staphylococcus aureus* after the introduction of an automatic milking system

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Summary

Staphylococcus aureus (*S. aureus*) is often the cause of mastitis problems in dairy herds and causes great economic losses. In this study, isolates from a dairy herd with a known *S. aureus* mastitis problem were examined by means of molecular methods (*spa* typing, PFGE, and DNA microarray) to investigate their epidemiological relationship and the success of intervention measures. The investigated dairy farm has a herd size of 60 cows and uses a fully automated milking system for milk production. A *S. aureus* strain, which contaminated the automated milking system and was subsequently spread among the herd through the latter, was suspected to be the origin of the mastitis problem within the herd. Thanks to the applied molecular methods, the common origin of the *S. aureus* isolates from the collected milk and swab samples could be shown. By culling chronically infected cows, optimising dry cow management and ensuring reliable intermediate cluster disinfection, the bulk milk somatic cell count improved.

Keywords: *Staphylococcus aureus*, bovine mastitis, automatic milking system, molecular epidemiology, genotyping

Erfolg von Interventionen bei Mastitiden mit *Staphylococcus aureus* als Herdenproblem nach der Installation eines automatischen Melksystems

Staphylococcus aureus (*S. aureus*) ist bei Milchkühen häufig die Ursache von Mastitiden auf Bestandsebene und führt zu grossen wirtschaftlichen Verlusten. In dieser Studie wurden Isolate eines Milchkuhbetriebs mit einem nachgewiesenen *S. aureus* Mastitis-Problem mittels molekularer Methoden (*spa* typing, PFGE, DNA microarray) untersucht um epidemiologische Zusammenhänge aufzuzeigen und den Erfolg der ergriffenen Massnahmen zu eruieren. Der untersuchte Betrieb hat einen Bestand von 60 Milchkühen und setzt einen vollautomatisierten Melkroboter zur Milchgewinnung ein. Als Ursprung des Mastitis Problems im Bestand wurde ein *S. aureus* Stamm vermutet, welcher den Melkroboter kontaminiert hatte und durch diesen im Bestand verbreitet wurde. Mittels der in dieser Studie angewandten molekulardiagnostischen Methoden wurde der gemeinsame Ursprung der aus den Milch- und Umgebungs-Tupferproben gewonnenen *S. aureus* Isolate gezeigt. Durch die Ausmerzungen chronisch infizierter Kühe, ein intensivierte Trockenstellmanagement und die Sicherstellung einer verlässlichen Melkzeug-Zwischendesinfektion konnte die Tankmilchzellzahl verbessert werden.

Schlüsselwörter: *Staphylococcus aureus*, Mastitis, Melkroboter, molekulare Epidemiologie, Genotypisierung

DOI 10.17236/sat00012

Received: 23.06.2014

Accepted: 21.08.2014

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most important causes of bovine mastitis and is associated with great economic losses in dairy herds (Hummerjohann et al., 2014). It colonizes the skin and skin lesions of animals and human beings (Petersson-Wolfe et al.,

2010). *S. aureus* mastitis is often subclinical initially, causing an increased somatic cell count (SCC) (Petersson-Wolfe et al., 2010). Therapy is challenging and often not successful, due to a multitude of strategies of the organism to evade the immune system, e.g. reverse binding of antibodies on its surface or hiding intracellularly in neutrophils or other host cells. Furthermore, a

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S. aureus induced mastitis can lead to toxic mastitis, which is characterized by a combination of mastitis symptoms and other clinical signs of toxæmia (Rüeggsegger et al., *in press*) with lethal outcome for the affected animal. The organism's ability to persist in mammary glands, teat canals and teat lesions of a subclinically infected cow among a healthy herd therefore leads to a massive threat to herd health. Transmission may in particular occur during the milking process from animal to animal through contact with contaminated milk residues in the milking machine. In addition, vectors such as flies favour the spread of *S. aureus* (Petersson-Wolfe et al., 2010). As clinical treatment alone is ineffective to control *S. aureus* infections in dairy herds, prevention of new infections and culling of infected animals is still the most promising way to solve this problem (Petersson-Wolfe et al., 2010). Thus, prevention of new infections remains essential. For this purpose milk machines or automated milking systems (AMS) require preventive maintenance on a regular schedule (Petersson-Wolfe et al., 2010). The aim of this study was to determine the relationship of isolates from a dairy farm with diagnosed *S. aureus* mastitis infection and to monitor the effect of the measurements implemented.

Animals, Material and Methods

Dairy farm

The presented dairy farm is situated in Liechtenstein on flatland, near the river Rhine. The farm livestock counts up to 60 dairy cows composed of Brown Swiss, Red Holstein and Holstein Friesian with a yearly milk yield of 8'700 kg per animal. In November 2011, the husbandry was changed from tethering to keeping the cows in a free stall barn. On this occasion an AMS was installed. Although strongly recommended by the manufacturer to exclude dairy cows with mastitis when starting to use the AMS, the farm manager left one dairy cow with a diagnosed *S. aureus* mastitis within the herd and to be milked by the AMS.

Udder health

The bulk milk somatic cell count (BMSCC) in the year before installation of the AMS was nearly $350 \times 10^3/\text{ml}$ and therefore at the upper limit determined by the legislative milking regulation. Between November 2011 and November 2013 the mean BMSCC remained stable compared to the values before installation of the AMS. However, the limit of $350 \times 10^3/\text{ml}$ was exceeded three times during this period of time and the BMSCC measurements indicated a greater irregularity with peaks of over $700 \times 10^3/\text{ml}$ and low values of approximately $150 \times 10^3/\text{ml}$. After exceeding a BMSCC of $350 \times 10^3/\text{ml}$ for the fourth time consecutively in August 2013, an inspection of the livestock and the AMS was performed

by a veterinarian. A California mastitis test (CMT) was performed for every cow in lactation ($n = 56$), with 35 cows showing at least one positive quarter (63%). Of the 35 milk samples, which were obtained from the CMT positive cows, 31 were positive for *S. aureus* (89%).

Representatives of the Division of Ambulatory Service and Herd Health were then involved in further analysis of the herd problem. At that time (September 2013), 79% of the cows (46 of 55) had an individual somatic cell count (SCC) over 150×10^3 cells/ml and the prevalence of *S. aureus* infections within the herd was 71% (39 of 55 cows). The investigations indicated that the main problems were located in the management of drying off cows, as well as in the management of the AMS. To this date, no systematic dry cow therapy was performed and chronically infected animals were neither treated nor culled. Furthermore, the AMS was not maintained as recommended by the manufacturer's instructions to check the steam disinfection daily and the steam disinfection proofed unreliable. To monitor the success of the measures and to determine if a single strain was at the origin of the herd problem, milk samples of all lactating cows were collected aseptically three times over a period of four months (September 2013, December 2013, and January 2014; sampling series one to three). Moreover, swab samples of different areas of the AMS were collected. In the following three months, milk samples of the freshening cows were investigated.

Microbiological methods

A total of 696 samples were collected over seven months of surveillance as shown in Table 1. Each sample was screened for *S. aureus* on a chromogenic medium (chromID® *S. aureus*, bioMérieux SA, Marcy l'Etoile, F: SAID) after 24 hours of incubation at 37°C. To sequence the polymorphic X region of the *spa* gene of the isolates, the protocol as previously described by Aires-de-Sousa et al. (2006) and Johler et al. (2011) was applied. The amplicons were sent to Microsynth (Balgach, CH) for sequencing. The obtained repeat sequences were then compared to known *spa* types on the *spa* server (<http://www.spaserver.ridom.de/>). Pulsed-field gel electrophoresis (PFGE) was performed according to Bannerman et al. (1995), with minor modifications. *Salmonella* serotype Braenderup (H9812) was used as a size standard. For the microarray-based genotyping the Genotyping Kit 2.0 (Alere, Jena, DE) following the manufacturer's instructions was applied. The samples were profiled by the platform ArrayMate Reader.

Results

Of the collected 696 samples a total of 227 milk samples and 26 samples from the AMS showed presumptive *S. aureus* positive colonies after incubation on a chromogenic medium as shown in Table 1. In total, 18 iso-

Table 1: Overview of the samples taken and of the results from the screening on a chromogenic medium (AMS: automated milking system).

	Origin of sample	Number of isolates	Presumptive <i>S. aureus</i> positive samples	% presumptive <i>S. aureus</i> positive samples
Sampling serie 1	Milk	124	85	69
		AMS	21	71
Sampling serie 2	Milk	250	82	33
		AMS	14	71
Sampling serie 3	Milk	234	57	24
		AMS	5	20
Follow-up period	Milk	48	3	6
		AMS	0	NA

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lates distributed over all three sampling series and from both cows and the AMS were selected for *spa* typing. Fifteen of them were assigned to *spa* type t2953 and three to the newly described *spa* type t13496. Eight of these isolates were selected for PFGE. Seven (all *spa* type t2953) of eight isolates showed a highly similar pattern, whereas one isolate (*spa* type t13496) differed from the main pattern, showing several bands at the size of approximately 138.9 kilobases and a difference in size of the digested DNA fragments between band size of 54.7 kilobases and 78.2 kilobases. Additionally, a total of eight isolates were further genotyped by a DNA microarray. All isolates were assigned to the clonal complex 8 (CC8) and harboured *agrI*, as well as the enterotoxin genes *sed*, *sej*, and *ser*.

Discussion

The present study revealed that the isolates from the collected milk and swab samples of the investigated dairy farm were closely related. This finding was confirmed by all of the three applied typing methods on selected isolates over all three sampling series.

The results of the *spa* and the PFGE typing suggest that the main *S. aureus* spreading on the described farm originated from one single strain. Interestingly, in every sampling series the isolates collected from the AMS exhibited the same *spa* type, corresponding to the most commonly discovered *spa* type (t2953) among the *S. aureus* isolates collected from the herd. The newly described *spa* type, t13496, was isolated from two dairy cows. The finding of only two different *spa* types within the herd is consistent with the hypothesis of a single strain causing the mastitis problem. Interestingly, the repeat sequence of *spa* type t13496 and *spa* type t2953 reveal a remarkable similarity: Seven repeats are identical, only three adjacent repeats are missing in t13496, suggesting that strains of these *spa* types may be closely related.

Seven of the eight isolates were positive for the enterotoxin *sea*, which is a common finding in *S. aureus* genotype B (GTB) strains (Fournier et al., 2008; Graber et al., 2009). Furthermore, *S. aureus* isolates belonging to clonal complex 8 and *spa* type t2953 are a common finding in mastitis milk in Swiss dairy herds as described by Johler et al. (2011). The association of the typed isolates to GTB and the fact that the examined isolates belong to the same strain is consistent with the mastitis herd problem in the investigated farm.

The counter-measures taken against the problem with *S. aureus* were effective. Concerning the AMS, the steam disinfection was insufficient, confirmed by swab samples that still yielded positive results for *S. aureus*. This certainly contributed to the dissemination of the bacteria. To overcome this problem, the disinfection procedure of the AMS was finally changed from hot steam to peracetic acid with intensive rinsing after the disinfecting procedure. To further decrease the infectious pressure, the farmer culled 14 chronically infected cows between the first and the third sampling period. To restock the herd, he bought in the same time frame eleven heifers and dry cows. All cows drying off were consequently treated with an antibiotic dry cow therapy product containing cloxacillin, factoring in antimicrobial sensitivity. The positive impact of this measure is displayed in the results of the follow-up period. All the *S. aureus* positive samples found during that period originated from cows dried off without antibiotics before the intervention. In summary, not only the prevalence of *S. aureus* infected cows declined drastically after implementing the mentioned preventive measurements, but also the percentage of cows with SCC over 150×10^3 cells/ml was declined from 79% in September 2013 to 62% in December 2013 and to 48% in January 2014.

Acknowledgements

We thank Henna-Maria Sihto for her support in the laboratory work.

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Succès des interventions lors de mammites à *Staphylococcus aureus* en tant que problème d'exploitation après installation d'un système de traite automatique

Le *Staphylococcus aureus* (*S. aureus*) est fréquemment cause de mammites en tant que problème d'exploitation chez les vaches laitières et il provoque de graves pertes économiques. Dans la présente étude, on a analysé des isolats provenant d'une exploitation laitière avec un problème de mammites à *S. aureus* par des techniques moléculaires (*spa* typing, PFGE, DNA microarray), afin de démontrer les relations épidémiologiques et le succès des mesures prises. L'exploitation examinée a un effectif de 60 vaches laitières et utilise un système de traite entièrement automatique. On suppose que l'origine du problème de mammites dans cette exploitation se trouve dans une souche de *S. aureus* qui aurait contaminé le robot de traite et qui se serait répandue par son intermédiaire dans toute l'exploitation. On a pu démontrer, au moyen des techniques de diagnostic moléculaires utilisées dans cette étude, l'origine commune des *S. aureus* isolés dans les écouvillons de lait et de l'environnement. Grâce à l'élimination des vaches chroniquement infectées, à une gestion intensive des tarissements et à l'assurance d'une désinfection intermédiaire du matériel de traite, on a pu réduire le nombre de cellules du lait de mélange.

Il successo degli interventi sulla mastite da *Staphylococcus aureus* come problema susseguente l'instal- lazione di un sistema di mungitura automatica

Lo *Staphylococcus aureus* (*S. aureus*) è spesso la causa nelle mucche da latte di mastite a livello della mandria e porta a perdite economiche notevoli. In questo studio, gli isolati di un'azienda di allevamento di mucche da latte con un problema di mastite da *S. aureus* comprovato da metodi molecolari (tipizzazione *spa*, PFGE, DNA microarray) sono stati esaminati per identificare le relazioni epidemiologici e per misurare il successo delle misure adottate. L'azienda analizzata possedeva una mandria di 60 mucche da latte e impiegava un robot di mungitura completamente automatizzato per la produzione di latte. Si sospetta che l'origine del problema della mastite nella mandria sia dovuto ad un ceppo di *S. aureus* che ha contaminato il robot di mungitura e tramite questo si è diffuso nella mandria. I metodi di diagnostica molecolare impiegati in questo studio hanno dimostrato l'origine comune dei tamponi derivati dal latte e dall'ambiente circostante degli isolati di *S. aureus* raccolti. Con l'eliminazione delle mucche infettate in modo cronico, una intensiva gestione della messa in asciutta e la garanzia di una disinfezione attendibile degli aspiratori si è ottenuto un conteggio migliorato del numero di cellule somatiche.

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